PROPOSAL FOR TOPIC APPROVAL TO BE SUBMITTED TO THE UNIVERSITY OF MUMBAI FOR DOCTOR OF PHILOSOPHY (SCIENCE) DEGREE IN THE SUBJECT OF BIOTECHNOLOGY

Title of the topic	Study of biodegradation of microplastics by native microorganisms.
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Date: 14/08/2023

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Study of biodegradation of microplastics by native microorganisms.

1. Aims of the study

1) Study of microplastics from various environmental samples like mangrove sediments, wastewater, beach sediments, etc.
2) Study of low-density polyethylene (LDPE) microplastic degradation by native microorganisms.
3) Insights into the probable mechanism of low-density polyethylene (LDPE) microplastic degradation by the native microorganisms.

2. Objectives of the study

1) Qualitative and quantitative analysis of microplastics in various environmental samples such as mangrove sediments, wastewater, beach sediments, etc.
2) Isolation and characterization of native microorganisms capable of degrading LDPE microplastics.
3) Evaluation of LDPE microplastic degradation efficiency of isolated native microorganisms.
4) Selection of highly efficient native microorganisms for LDPE microplastic degradation and developing the microbial consortia for efficient microplastic degradation.
5) Studying the various degraded by-products of the degradation of LDPE microplastics by microorganisms.
6) Understanding the probable mechanism of LDPE microplastic degradation by microorganisms using molecular approaches such as proteomics to study the differential expression of proteins/enzymes.

3. Study background and significance of the study

Plastics are widely used in various sectors due to their exceptional mechanical properties, low cost, lightweight, stability, durability, and chemical inertness. However, their extensive production has led to a significant environmental concern owing to their low degradability, which could result in persistent accumulation and pollution for centuries. (Raddadi et al., 2019). In the last seven decades, plastic production has soared to an astounding 359 million metric tons worldwide, primarily centered on the manufacturing of consumer products and packaging materials (Adhikari et al, 2021) Plastics that are disposed of in landfills are often exposed to photo-oxidation leading to the small fragments known as microplastics (Raddadi et al., 2019).

According to the UN environment program report, microplastics are minute particles with an upper limit of 5mm diameter. In addition to landfills, microplastics found in the marine environment can originate from the fragmentation of larger plastic fragments and various land sources such as tire and road wear particles, laundry, household dust, and personal care products (Raddadi *et al.*, 2019).

Due to their widespread use in various industries and mass production, microplastics have significantly contributed to environmental pollution. Their abundance has exponentially increased leading to a severe environmental issue (Habib *et al.*, 2020). Around 80% of marine litter is comprised of plastics, and of that amount, roughly 90% consists of microplastics (Rad *et al.*, 2022). Microplastics account for 92.4% of plastic waste and are primarily composed of polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinylchloride (PVC), nylons, polylactic acid, polyamide and polyethylene terephthalate (PET) (Auta *et al.*, 2017). Out of all the plastic types polyethylene (PE) has the maximum worldwide consumption of about 30% in the year 2018 (Adhikari et al, 2021) They are found in various shapes such as fibres, fragments, and spheres, with fibres being the most commonly reported form (Wright *et al.*, 2017).

Microplastics are a major concern due to their surface morphology, small particle size, high abundance, and hydrophobic nature, which enables them to absorb and transport various contaminants (Bhatt *et al.*, 2021). They also adsorb and accumulate heavy metals such as cadmium, zinc, nickel, and lead. Microplastics may also have some endogenous chemicals incorporated during the manufacture of plastic products. These chemicals can leach into any external medium. Microplastics are reportedly present in seafood, processed food items, etc. Humans are exposed to microplastics through inhalation of indoor air, direct swallowing of house dust or house dust settling on food, or exposure to the particles released from plastic food containers or bottles (Wright *et al.*, 2017). Microplastics, due to their tiny size, are easily consumed by organisms at all levels of the food chain. Because of their composition and large surface area, they tend to attract waterborne organic pollutants and leach toxic plasticizers. When organisms ingest microplastics, they may be exposing themselves to toxins at the bottom of the food chain, which could accumulate over time (bioaccumulation) (Cole *et al.*, 2011).

Microplastics find their way into the human body through various pathways, including ingestion of contaminated food and beverages, inhalation of airborne particles, and direct dermal contact with products, textiles, or dust containing microplastics. (Prata et al., 2019) The annual intake of microplastics per person, estimated from food consumption, varies from

39,000 to 52,000 particles, while through inhalation, it ranges from 35,000 to 62,000 particles. (Cox et al., 2019 Based on a recent study examining plastic fragments in human placentas, it is indicated that exposure to microplastics may initiate before birth. (Ragusa et al., 2020) Microplastics (MPs) have the potential to accumulate within the human body, leading to damage in various tissues and inducing chronic inflammation. Additionally, MPs can enter the bloodstream, as supported by recent research, and are shown to accumulate in tissues like the liver, kidney, and gut of mice, thereby influencing metabolism and oxidative stress levels. (Bhatt et al., 2021)

Numerous researchers have studied the biodegradation of plastics as an environmentally friendly and economically viable approach for their decomposition (Rad *et al.*, 2022). Microbial degradation appears to be the most feasible way to hasten the deterioration of microplastics. Specific microorganisms tend to colonise plastic polymers, initiating biodegradation by adhering to plastic surfaces via biofilm formation. Once colonised, these microbes secrete proteinases that catalyse the hydrolysis of the plastic polymers, resulting in the gradual formation of oligomers, monomers, and metabolic intermediates which eventually mineralise into carbon dioxide and water molecules (Habib *et al.*, 2020). Hence, in the current study, it is proposed to use native microorganisms for the degradation of microplastics. Also, the current study is attempting to understand the mechanism of microplastic degradation by microorganisms. This will help in the long run to develop efficient and sustainable technology for the degradation of microplastics.

4. National status

A study conducted in Madhurai by (Devi *et al.*, 2021), where they demonstrated the biodegradation of LDPE and PP microplastics (pre-treated by UV) by using strains of *Bacillus cereus* and *Bacillus paramycoides* isolated from the Vaigai River. *Bacillus cereus* showed a degradation rate of 68% for PP microplastic and 63% for LDPE microplastic whereas *Bacillus paramycoides* showed a degradation rate of 79% for PP microplastic and 41% for LDPE microplastic in 21days. Another study by Pathak et al, 2023, where they studied the biodegradation of LDPE microplastics by using *Bacillus subtilis* strain V8, *Paracoccus aminophilus* strain B14-, *Pseudomonas putida* strain C 25, *Pseudomonas aeruginosa* strain V1 and *Acinetobactor calcoaceticus* strain V4 which showed percentage weight loss in the range of 15-20%. (Gopinath *et al.*, 2020), conducted a study to determine the quantity and types of microplastics present in sediment and water samples collected from Red Hill Lake in Chennai

city. The study revealed that the concentration of microplastics in the water samples was 5.9 particles/L, while the concentration in the sediment samples was 27 particles/kg. The majority of the recovered microplastics were of the polyethylene and polypropylene types. Additionally, the morphological analysis showed that the most prevalent microplastics were fibrous in shape and white in colour. In their study, (Mohan *et al.*, 2022) conducted a comprehensive analysis to determine the prevalence of meso and microplastics in beach sediments across various beaches in Port Blair, Andaman and Nicobar Islands. The study focused on three specific coastal stations: Cove Beach, Quarry Beach, and Wandoor Beach. At Wandoor Beach, the concentration of microplastics ranged from 105 to 475 particles per kilogram (MPs/kg). The predominant polymer type identified was polypropylene (PP), and the most commonly observed microplastics were in fibrous and line shapes. Quarry Beach exhibited microplastic concentrations ranging from 72.5 to 222.5 MPs/kg. The predominant polymer types detected were high-density polyethylene (HDPE), and styrene, among others. The prevalent microplastics at this site were in fibrous, line, and fragment shapes. Cove Beach demonstrated microplastic concentrations varying from 125 to 457 MPs/kg. The predominant polymer types identified were PP and polyethylene terephthalate. The most prevalent microplastics observed were fibrous, line, and fragment shapes.

5. International status

As microplastics are a significant contributor to environmental pollution (Habib *et al.*, 2020) its biodegradation is widely studied, such as,

In (Rad *et al.*, 2022), the researchers obtained *Achromobacter denitrificans* from compost and employed it for the biodegradation of PVC microplastic and LDPE microplastic that had undergone thermo-oxidative pre-treatment. The resulting weight loss was approximately 12.3% and 6.5% for PVC microplastic and LDPE microplastic respectively. Another study was conducted to check the biodegradation of polypropylene by bacteria isolated from Antarctic soil. In this study, (Habib *et al.*, 2020), isolated two bacterial strains namely *Pseudomonas sp*. ADL15 and *Rhodococcus sp*. ADL36. After 40 days of incubating the bacterial strain with polypropylene microplastics, ADL15 and ADL36 showed weight loss percentages of 17.3% and 7.3% respectively.

In a study conducted in Malaysia by (Auta *et al.*, 2018), the growth response and degradation mechanism of polypropylene microplastic using *Bacillus sp*. Strain 27 and *Rhodococcus sp*.

Strain 36, which was isolated from Mangrove sediments, was evaluated. The ability of both bacterial strains to use polypropylene microplastics for growth was confirmed by observing a decrease in polymer mass. After 40 days of incubation, *Rhodococcus sp*. Strain 36 showed a weight of 6.4% while *Bacillus sp*. The strain showed a weight loss of 4%.

In their study, (Murphy *et al.*, 2016), investigated a wastewater treatment plant (WWTP) located on the river Clyde in Glasgow. The researchers collected samples at different stages of the treatment process to assess the presence of microplastics. Four specific stages were sampled: influent after 19 mm coarse screening (S1), grit and grease effluent (S2), primary effluent (S3), and the final effluent (S4) before its release into the river Clyde.

The results revealed that S1 had a concentration of 15.70±5.23 microplastics per litre (MP/L). S2 showed a concentration of 8.70±1.56 MP/L, S3 showed 3.40±0.28 MPs/L, and S4 showed 0.25±0.04 MPs/L. These findings indicate that the amount of microplastics decreases at each stage of the wastewater treatment process. However, when considering the average outflow of the wastewater plant, extrapolation suggests that approximately 64±11 million microplastics are still released daily into the receiving water body. This highlights the importance of addressing microplastic pollution despite the reduction achieved through the treatment process.

6. Study hypothesis

Microplastics are a threat to the environment and there is an urgent need to remediate the microplastic from the environment. Monitoring the presence of microplastics in various environmental samples is important to understand the intensity of microplastic pollution. The current study proposes a qualitative and quantitative analysis of microplastics to understand the extent of microplastic pollution in various environmental samples and establish a baseline for it. In the current study, the native microorganism will be used for microplastic degradation, which is an environmentally safe and sustainable solution for microplastic degradation. Various studies have indicated the capacity of microorganisms to degrade microplastics. Certain native microorganisms, isolated from diverse environmental sources such as soil, water bodies, and sediments, exhibit distinct enzymatic capabilities and adaptations for effective biodegradation of microplastics. It is hypothesized that these microorganisms will demonstrate varying degrees of microplastic degradation efficiency and the study aims to investigate the biodegradation potential of these microorganisms. By analyzing the changes in microplastic mass, surface morphology, and change in functional groups over time, the study seeks to assess

the efficiency of microbial biodegradation in reducing microplastic pollution. Moreover, the research will explore the role of specific enzymes/proteins through the proteomics approach involved in the breakdown of different types of microplastics. The findings from this study will contribute to a deeper understanding of microbial microplastic degradation as a strategy for reducing the impact of microplastics on the environment and understanding the extent of actual microplastic pollution in our environment.

7. Study rationale

Microplastics, as a widespread environmental pollutant, pose significant threats to ecosystems and organisms. The lack of an established management system for microplastics calls for approaches, such as bioremediation, to tackle this issue. Although some information on microplastic degradation exists, it is important to recognize that microorganisms isolated from different environments may exhibit varying abilities to degrade microplastics. Additionally, the absence of standardized protocols for qualitative and quantitative microplastic measurements hinders the establishment of baseline pollution levels. Furthermore, the mechanisms involved in microplastic degradation by microorganisms remain poorly understood. This study aims to investigate the potential of microorganisms in biodegrading microplastics, providing valuable insights into the feasibility and efficacy of biodegradation as a strategy to mitigate the environmental and health impacts of microplastic pollution.

8. Research methodology:

1) Sample collection:

a) For the isolation of microplastic degrading micro-organisms, samples will be collected from soil/mangrove sediment/ water samples. All samples will be collected in triplicates.

b) For quantification of microplastics in various environmental samples such as wastewater, mangrove sediments, beach sediments, etc. the sampling process will be followed as mentioned in the Microplastic sampling guidelines by Mississippi State University.

2) Qualitative and quantitative analysis of microplastics:

   Various environmental samples such as soil, wastewater, mangrove sediments, etc. will be evaluated for microplastics and quantification of various types of microplastics such as LDPE, polyethylene (PE), polypropylene (PP), polystyrene (PS), polyvinylchloride (PVC), etc. will be done using various techniques such as optical microscopy/FTIR, etc.

3) Enrichment and isolation of microplastic degrading micro-organisms:

The samples will be added to Bushnell-Hass media/Mineral Salt media which contains LDPE microplastics as the only source of carbon. They will be incubated for a few days. After the enrichment process, the micro-organisms will be isolated on media. The process will be carried out as mentioned in Rad et al, 2022 and Auta et al, 2017.

4) Identification and characterisation of isolated micro-organisms.

   The isolated organism will be subjected to various biochemical tests such as to help determine its likely identity. The molecular identity of the isolated micro-organism will be conducted by using 16S rRNA technology. This region is conserved in bacteria and can be used to identify it.

5) Microbial consortium development.

   The microbial consortium for microplastic degradation will be developed by growing the singly isolated micro-organism in various combinations. The bacterial combination that can efficiently degrade microplastics will be selected and utilized as the bacterial consortium for LDPE microplastic degradation.

6) Understanding the biodegradation process through various techniques:

   The biodegradation study will include examining the changes in the chemical structure and physical properties of LDPE microplastic when incubated with the isolated micro-organisms.

a) To assess the biodegradation of LDPE microplastics and their physical property changes, the following methods will be utilized:

- Weight loss measurement: the weight loss of LDPE microplastics will be measured after incubating them with the micro-organisms.
- Changes in the functional group due to microbial attack can be detected by FTIR etc.
- Scanning electron microscopy observations: Variations in the surface topography of LDPE microplastics will be examined.
- Secondary products will be identified using GC-MS etc.

b) To study different aspects of the microbial population during the biodegradation process, the following methods will be utilized:

- Plate count method will be used for monitoring microbial growth and viability throughout the incubation period.
- Protein content of the bacteria will be done by techniques such as Folin-Lowry method or Bradford method etc.
- Bacterial cell surface hydrophobicity will be evaluated by assays such as

BATH etc.

- Scanning electron microscopy observation for observing the microbial population that adheres to microplastics.

7) Probable mechanisms of biodegradation through proteomics:

To understand the protein and enzymes involved in the biodegradation of LDPE microplastics, protein profiling will be conducted. The isolated microorganisms will be grown and incubated with LDPE microplastics. Proteins will be extracted from the micro-organisms that degrade the microplastic and will be quantified using a standardised method such as Folin-Lowry, Bradford method, etc. Difference protein expression will be analysed by separating the extracted proteins on SDS PAGE, comparing those present in the presence of LDPE microplastics and present in the absence of LDPE microplastics. Mass spectroscopy technique will be utilised for the identification of the protein.

9. Statistical plan

1) The data obtained from conducting tests in triplicates will be presented as mean values with their corresponding standard deviations.
2) To access the varying abilities of different microorganisms in microplastic degradation, the percentage weight loss of microplastics by each microorganism and the growth rates of the microorganisms will be analysed using ANOVA/t-test/Mann-Whitney test, etc.
3) By using statistical tests such as ANOVA/Kruskal-Wallis test etc, the differences in the abundance and types of microplastics among various sources.

10. Limitations

The study may be limited by:

1) The laboratory conditions used to study microplastic biodegradation may differ from actual environmental conditions.
2) In the laboratory, the microplastic degradation study will be done for a limited time e.g. 15, 30, 45 days, etc. whereas, in the natural environment, this process will be for longer times e.g. for months, or years.
3) The presence of a variety of organic and inorganic compounds in environmental samples such as soil/landfill/wastewater may interfere with the biodegradation process.

4) The study may focus on a specific group of microorganisms or isolate strains from certain environments, potentially overlooking the biodegradation potential of other microorganisms present in different ecosystems.

11. Expected outcomes

1) Identification and quantification of different types of microplastics present in various environmental samples such as mangrove sediments, waste water, beach sediments etc.
2) Identification and isolation of micro-organisms capable of effectively degrading LDPE microplastics from different sources.
3) Characterization of the degradation process of LDPE microplastics and the structural changes that occur during the process.
4) Development of a microbial consortium at a laboratory scale to enhance the degradation of LDPE microplastics.
5) Various by-products of degradation of LDPE microplastics will be identified.
6) Determination of the potential mechanism for LDPE microplastic degradation by the isolated micro-organisms through proteomics.

References

1) Adhikari, Sangeet, et al. "Methods and challenges in the detection of microplastics and nanoplastics: a mini-review." *Polymer International* 71.5 (2022): 543-551.
2) Auta, Helen Shnada, et al. "Growth kinetics and biodeterioration of polypropylene microplastics by Bacillus sp. and Rhodococcus sp. isolated from mangrove sediment." *Marine Pollution Bulletin* 127 (2018): 15-21.
3) Bhatt, Pankaj, et al. "Microplastic contaminants in the aqueous environment, fate, toxicity consequences, and remediation strategies." *Environmental Research* 200 (2021): 111762.
4) Cole, Matthew, et al. "Microplastics as contaminants in the marine environment: a review." *Marine pollution bulletin* 62.12 (2011): 2588-2597.
5) Gopinath, Kalpana, et al. "Quantification of microplastic in red hills lake of Chennai city, Tamil Nadu, India." Environmental science and pollution research 27 (2020): 33297-33306.
6) Habib, Syahir, et al. "Biodeterioration of untreated polypropylene microplastic particles by Antarctic bacteria." *Polymers* 12.11 (2020): 2616.
7) Miri, Saba, et al. "Biodegradation of microplastics: Better late than never." *Chemosphere* 286 (2022): 131670.
8) Mohan, P. M., et al. "A baseline study of meso and microplastic predominance in pristine beach sediment of the Indian tropical island ecosystem." *Marine Pollution Bulletin* 181 (2022): 113825.
9) Nanthini Devi, K., et al. "Biodegradation of low-density polyethylene and polypropylene by microbes isolated from Vaigai River, Madurai, India." *Archives of Microbiology* 203 (2021): 6253-6265.
10) Patchaiyappan, Arunkumar, et al. "Occurrence, distribution and composition of microplastics in the sediments of South Andaman beaches." *Marine Pollution Bulletin* 156 (2020): 111227.
11) Pathak, Vinay Mohan. "Exploitation of bacterial strains for microplastics (LDPE) biodegradation." *Chemosphere* (2023): 137845.
12) Prata, Joana Correia, et al. "Methods for sampling and detection of microplastics in water and sediment: a critical review." *TrAC Trends in Analytical Chemistry* 110 (2019): 150-159.
13) Rad, Mojtaba Maleki, Hamid Moghimi, and Ehsan Azin. "Biodegradation of thermo-oxidative pretreated low-density polyethylene (LDPE) and polyvinyl chloride (PVC)

microplastics by Achromobacter denitrificans Ebl13." *Marine Pollution Bulletin* 181 (2022): 113830.

14) Raddadi, Noura, and Fabio Fava. "Biodegradation of oil-based plastics in the environment: Existing knowledge and needs of research and innovation." *Science of the Total Environment* 679 (2019): 148-158.

15) Tang, Kuok Ho Daniel, and Tony Hadibarata. "The application of bioremediation in wastewater treatment plants for microplastics removal: a practical perspective." *Bioprocess and Biosystems Engineering* 45.11 (2022): 1865-1878.

16) Wright, Stephanie L., and Frank J. Kelly. "Plastic and human health: a micro issue?." *Environmental Science & Technology* 51.12 (2017): 6634-6647.

17) Murphy, F., Ewins, C., Carbonnier, F., & Quinn, B. (2016). Wastewater Treatment Works (WwTW) as a Source of Microplastics in the Aquatic Environment. Environmental Science and Technology, 50(11), 5800–5808. https://doi.org/10.1021/acs.est.5b05416

18) Prata, Joana Correia, et al. "Environmental exposure to microplastics: An overview on possible human health effects." Science of the total environment 702 (2020): 134455.

19) Cox, Kieran D., et al. "Human consumption of microplastics." Environmental science & technology 53.12 (2019): 7068-7074.

20) Ragusa A, Svelato A, Santacroce C, Catalano P, Notarstefano V, Carnevali O, Papa F, Rongioletti MCA, Baiocco F, Draghi S, D'Amore E, Rinaldo D, Matta M, Giorgini E. Plasticenta: First evidence of microplastics in human placenta. Environ Int. 2021 Jan;146:106274. doi: 10.1016/j.envint.2020.106274. Epub 2020 Dec 2. PMID: 33395930.